Reference: *Biol. Bull.* **194**: 1–6. (February, 1998)

Mechanical Significance of Obliquely Striated Architecture in Nematode Muscle

A. H. JAY BURR* AND CARL GANS

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada; and Department of Zoology, University of Texas, Austin, Texas 78746

In certain invertebrate muscles, adjacent narrow columns of sarcomeres are displaced along the fiber axis, providing an obliquely striated myofilament pattern in certain section planes. Although this architecture is described in many phyla and has been the subject of much discussion (1–12), its mechanical significance has yet to be resolved. In nematodes, where ultrastructural details of the obliquely striated muscle have long been known (12–19), another unique and prominent feature is the attachment of every sarcomere to the plasmalemma and basal lamina via dense bodies (Z-disc analogs). Unfortunately, the importance of this feature to the transmission of the contractile force to the cuticle is not understood outside the Caenorhabditis elegans *literature: it was overlooked in recent reviews covering obliquely striated muscle (9–11). Here we consider transmission of force and oblique striation together. We compare the contractile architecture in* C. elegans *with that in the more complex muscle type of larger nematodes. Both types are designed to transmit the force of contraction laterally to the cuticle rather than longitudinally to the muscle ends. In the second type, folding of the contractile structure around an inward extension of the basal lamina enables a higher number of sarcomeres to be linked to cuticle per unit length. We suggest that the mechanical significance of the oblique arrangement of sarcomeres in both types is that it distributes the force application sites of the sarcomeres more evenly over the basal lamina and cuticle. With this muscle architecture, smooth bending of the nematode body tube would be possible, and kinking would be prevented.*

In nematodes, four bands of muscles lie longitudinally along the cuticle. Bending is caused by compression of the cuticle due to contraction of either the dorsal or ventral muscle pairs with opposing action from the hydrostatic skeleton (20–21). The undulatory locomotion (see Fig. 2A) is due to smooth waves of bending that apply the propulsive forces to the substrate (22).

In all nematodes, the longitudinal muscle bands are composed of a single layer of cells, and the contractile filaments form a layer along the distal surface of each cell. The structure of the filament layer is diagrammed in Figure 1 for the two main types of muscle fiber architecture found in nematodes: platymyarian, represented here by *Caenorhabditis elegans*, and coelomyarian, represented by *Mermis nigrescens*. The structures of other platymyarian and coelomyarian nematode species are closely similar to these examples (12–15, 19).

In both types of architecture the myofilaments lie parallel to the long axis of the muscle fiber and the body axis (vertical in Fig. 1). Observe the one complete sarcomere shown in the frontal plane of the *Mermis* diagram and the partial ones in the other section planes. Clusters of thin filaments attach to each bar-shaped electron-dense structure. Called dense bodies or Z-bars, the latter are equivalent to the Z-discs of vertebrate cross-striated muscle in that they provide attachment sites for the actin filaments and form the boundaries between the narrow sarcomeres (16). Within each sarcomere, the thick filaments are linked to each other and to the plasmalemma by the M-line material (Fig. 1).

Probably the most significant and distinguishing structural feature of obliquely striated muscle of nematodes is the direct attachment of each dense body, and thereby each sarcomere, to the muscle cell membrane (Fig. 1). In cross-striated muscle there is no equivalent attachment—only a loose linkage of Z-discs with others along the same myofibril, between neighboring myofibrils, and to sites on the sarcolemma. These connections are made via

Received 12 May 1997; accepted 1 October 1997.

*To whom correspondence should be addressed. E-mail: burr@sfu.ca

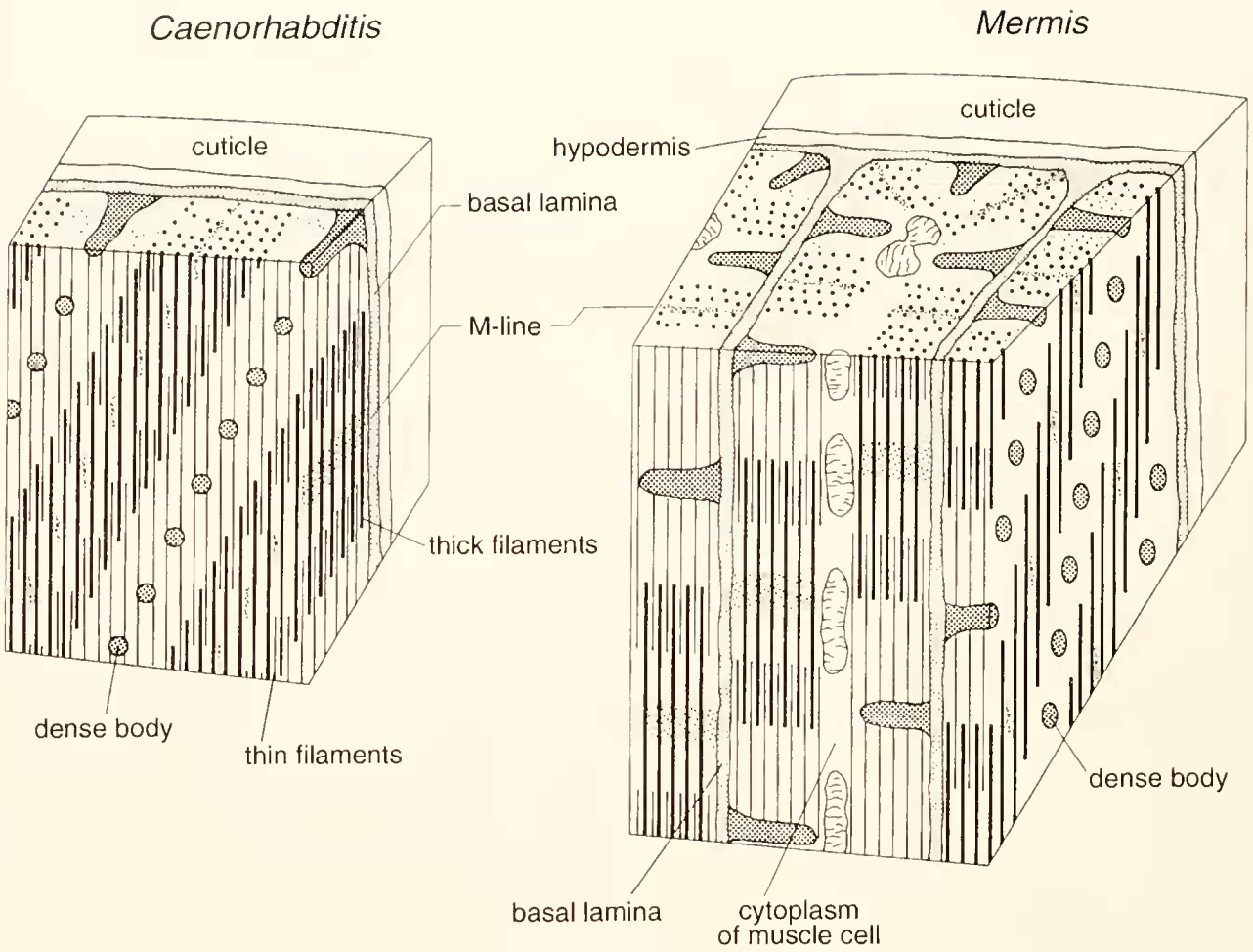

Figure 1. Contractile architecture of the two main types of nematode body-wall muscle: platymyarian (example: *Caenorhabditis elegans*) and coelomyarian (example: *Mermis nigrescens*). The anterior-posterior axis is vertical. For clarity, only two thin filaments of the bundle that attach to each dense body (13–14, 29) are illustrated in the tangential section of the *Caenorhabditis* diagram. In the equivalent, radial plane of the *Mermis* diagram, the thin filaments have been omitted altogether. The scale of the diagrams was shortened in the vertical direction to allow illustration of a complete sarcomere. *Methods:* The *C. elegans* structure was redrawn at a new orientation from illustrations by Francis and Waterston (16). That of *Mermis* was drawn from transmission electron micrographs of orthogonal sections (29).

an exosarcomeric lattice of intermediate filaments (23). The intermediate filament lattice can transmit forces along a myofibril, but only when a sarcomere is stretched beyond its normal operating range (24), and there is no evidence for lateral transmission of forces to the sarcolemma. In smooth muscle, also, there is no equivalent lateral attachment. The myofilament strands containing actin-myosin contractile units are anchored at their ends to the plasmalemma by membrane-associated dense bodies, or adherens junctions. However, cytoplasmic dense bodies that serially link contractile units within the strands are not directly attached to the plasmalemma (25–26).

By contrast, in nematode body wall muscle, all dense bodies appear firmly attached to the sarcolemma. Like the force-transmitting attachment plaques at the ends of cross-striated muscle cells, the electron-dense, α-actinin-containing dense bodies are fastened to the plasmalemma and basal lamina by a broad base composed of vinculin, talin, and integrin (16–18, 27). These components also play a role in sarcomere localization and assembly during embryonic development (27). The dense bodies are analogous to focal adhesions of vertebrate non-muscle cells in that they attach actin filaments through the plasmalemma to the basal lamina via the same protein components (28).

In the platymyarian type of nematode muscle cell, the contractile structure consists of a layer, about 1 μm in thickness, of myofilaments parallel to the very thin epidermis (hypodermis) and cuticle (Fig. 1). The ~1-μm dense

bodies traverse the entire depth of the filament layer and thereby attach the myofilaments perpendicularly to the sarcolemma and basal lamina. The basal lamina underlying the muscle, in turn, is fastened to the cuticle across the 0.15-μm-thick epidermal cell cytoplasm via 1-μm-spaced, 1-μm-wide circumferential bands of hemidesmosomes and intermediate filaments (16–17). Thus, the ends of each sarcomere are tightly anchored to the cuticle via dense bodies, basal lamina, and hypodermal cytoskeleton, and the shortening of a sarcomere would compress the cuticle locally.

In the coelomyarian muscle type, seen in *Mermis nigrescens* (19, 29) and various other large nematodes including *Ascaris lumbricoides* (reclassified *suum*) (12–14), the plate of myofilaments is folded lengthwise so that most of the contractile layer lies radially (Fig. 1). The thickness of the myofilament plate is the same (1–2 μm) in *Mermis* and *C. elegans;* however, it is two times thicker in the much larger *Ascaris* (13). In the folded contractile layer of coelomyarian muscle, the dense bodies connect the thin filaments perpendicularly to the basal lamina, as in platymyarian muscle, but most of the basal lamina is in the form of a longitudinal ribbon that extends radially inwards between the cells (Fig. 1). This rearrangement markedly increases the number of sarcomeres that can be connected to a unit length of cuticle, thus increasing the maximum contractile force. These ribbons of basal lamina would transmit compressive forces radially to the circumferential basal lamina lining the hypodermis. There, as in the platymyarian arrangement, hemidesmosomes and intermediate filaments are present in the thin hypodermal cytoplasm (12, 30) which would transmit compressive forces across it to the cuticle.

Hence, the links between thin filaments and the cuticle are the same in the two types, but with the radial extension of the basal lamina in coelomyarian muscle. In both types, the density and thickness of the dense bodies suggest rigidity, and the increased electron density seen in the basal lamina adjacent to the dense bodies and in the cuticle near the intermediate filaments (12, 18–19, 29–30) suggests that these attachment sites are reinforced to distribute stress. In the coelomyarian type, the radial extension of the basal lamina would add an extra element of elasticity to the link between sarcomere and cuticle.

The M-line material in the center of the A band (Fig. 1) attaches the thick filaments to the basal lamina and thus could also transmit forces to the basal lamina; however, it is unlikely to contribute significantly to the compressive forces acting on the basal lamina. Nematode M-lines are similar in density and thickness to those in cross-striated muscle fibrils. The relatively thicker and more electron-dense dense bodies are more probably the primary force transmitters. Like dense bodies, the M-line material is attached via β-integin and talin, but vinculin and α-actinin are missing (16, 28). M-lines are thought to keep the thick filaments roughly in register and centered within the sarcomere. The forces transmitted via the M-line in nematode muscle are probably limited to what is needed to maintain the average position of the thick filaments in a sarcomere centered between the dense bodies.

A system of inelastic fibers that interconnect dense bodies along the oblique row in *Ascaris* (12) is now known to be intermediate filaments (30). These are coiled except in fully extended muscle, so their role—like that of the exosarcomeric intermediate filament lattice of cross-striated muscle—may be to limit over-extension (8, 12, 15, 30).

The mechanical linkage in cross-striated muscle cells is very different. The sarcomeres of vertebrate myofibrils are assembled into cylindrical columns, the myofibrils. Tensile forces are transmitted longitudinally by the end-to-end contacts across the Z-discs bounding the sarcomeres and across the attachment plaques connecting between cell ends. In contrast, the contractile apparatus of nematodes appears to be designed for transmitting compressive forces laterally to the cuticle. In *C. elegans*, the attachment sites of sarcomeres via dense bodies to the basal lamina are far more numerous than the attachment plaques at the ends of the cells. In *Mermis*, the lateral attachments to the basal lamina extend over the entire 6-mm length of the extremely narrow (2 μm) band of contractile filaments. These dimensions are to be compared to the 5-10 mm arc lengths of the smooth bends which are propagated along the body. These geometries suggest that comparatively little of the contractile force developed within the longitudinal muscles is transmitted serially (from sarcomere to sarcomere and cell to cell) to the muscle ends. Rather, shortening of each sarcomere would directly compress the adjacent basal lamina to which it is individually and tightly attached. From the basal lamina, this compression would be transmitted to the cuticle by the many short intermediate filaments traversing the 1.5-μm hypodermis. Thus, the most significant mechanical distinction between obliquely striated muscle of nematodes and cross-striated muscle is that each sarcomere is connected perpendicularly to the site of force application, rather than serially to the muscle ends.

The functional consequence of this lateral, parallel application of contractile force to the cuticle is evident in the motion caused by the longitudinal muscles. Nematode locomotion typically involves smooth dorsoventral bending of the body tube (Fig. 2A). Although the straps of longitudinal muscles run the entire length of the body, compression of the cuticle occurs locally, on the inside of bends, for two or more separated bends along the length. This could occur only if local, lateral application of contractile force was significant. Localized bending is

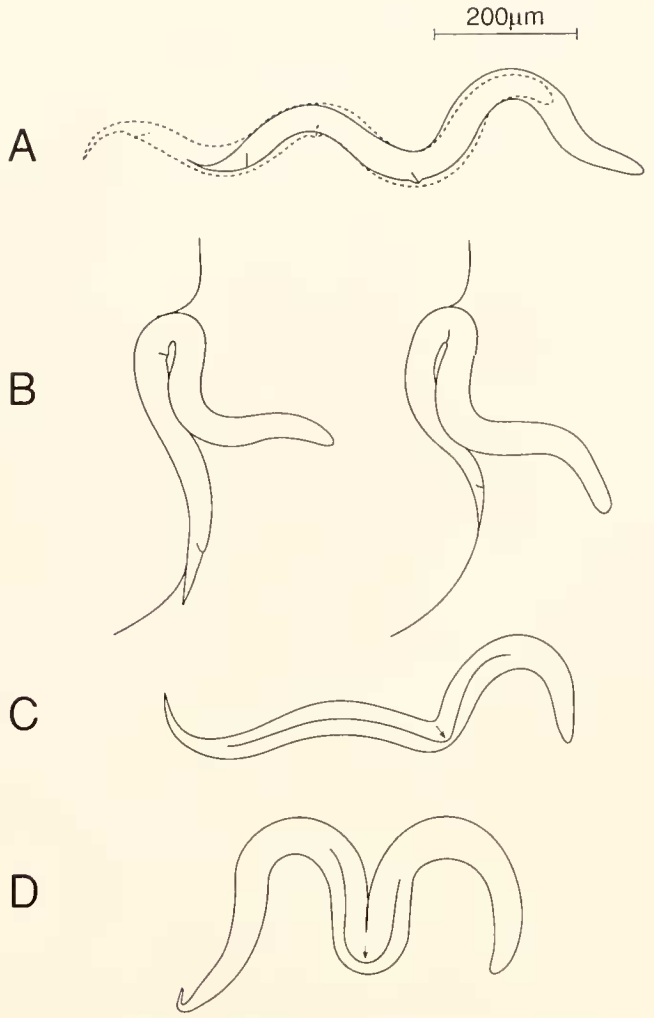

Figure 2. Propulsive motion of wild-type and mutant *Caenorhabditis elegans*. (A) Free forward motion of wild-type on surface of agar gel (two views 1.2 s apart). (B) Propulsion of wild-type in contact with surface of a bubble (two views 1.0 s apart). The external capillary force causes buckling of the body tube. Marks indicate location of vulva (midbody) and anus of this young adult hermaphrodite. There is some foreshortening due to out-of-plane orientation. (C) Kink that occurs during forward locomotion of a *lin-39* mutant of *C. elegans* crawling on agar. Dorsal is upwards. (D) Sharp kink that occurs during reverse locomotion of the same individual. Arrows point to the position of the refractile intestinal lumen, visible under the illumination conditions. *Methods:* Locomotory motion of *C. elegans* wildtype (Bristol, N2 strain), and the *lin-39* (n1760 null allele) mutant kindly provided by Scott Clark (32), was recorded on videotape with a CCD camera mounted at the focal plane of a 3.2 power microscope objective with diffused illumination from below. Body outlines were traced from a video monitor during frame-by-frame replay using a Mitsubishi Model HS-U62(C) video recorder. *C. elegans* strains were cultivated on agar petri plates with a culture of *Escherichia coli* mutant strain OP-50 for food (34).

especially evident in the unique locomotion of *Mermis*. Free-living stages of *Mermis nigrescens* (about 100 mm in length and 0.4 mm in diameter) are capable of remarkable local control of six to eight body waves so as to apply lateral force to various point contacts in grass as the body tube glides by (29).

The lateral attachment is also important to prevent buckling of the inherently flexible cuticle during contraction of the longitudinal muscles. Buckling would be predicted to occur if the muscle attachments were to span long intervals of the cuticle, in particular, if the ratio of length over width of the compressed zone exceeded 7, the value that shifts the Euler column formula into the long-column phase governed by buckling (31). The narrow cuticular tube of wild-type *C. elegans* buckles only when unusual external forces are applied—for example, when the worm becomes trapped by surface tension against a bubble (Fig. 2B). However, in the presence of the *lin-39* (n1760) mutation, buckling occurs during normal locomotion. The mutation leaves a substantial length of the ventral muscle detached from the cuticle in the midbody region (32). The detached sarcomeres remain interconnected serially like vertebrate myofibrils, through dense bodies and attachment plaques between cells. During contraction, the muscle strand applies forces to the cuticle anterior and posterior to the midbody region. When these mutants locomote, the body tube is seen to buckle as the wave of contraction passes into the midbody (Fig. 2C and 2D). Kinking is greatest in dorsal bends where the detached ventral muscle bands could slip the farthest across the pseudocoelom from their normal position, thus producing a greater component of force perpendicular to the cuticle. Arrows indicate displacement of the intestine (refractile lumen) by the muscle. Mutations in the *mua* (muscle attachment) genes similarly result in muscle detachment and kinking (Plenefisch and Hedgecock, pers. comm.).

Oblique striations are observed in longitudinal sections of *C. elegans* and radial sections of *Mermis* (Fig. 1). The oblique pattern is due to the displacement of adjacent columns of sarcomeres along one of the longitudinal dimensions. The actual angle of oblique striation could not be shown in Figure 1 but is seen in Figure 3A, a diagram illustrating to scale a surface view of the basal lamina and the dense body attachment sites. In all nematodes described the striation angle is 5°–7° with respect to the myofilaments (13–14, 16, 33) and depends on degree of contraction (12).

Contraction of obliquely striated muscle occurs by a sliding filament mechanism. But unlike cross-striated muscle, the angle of striation with respect to the filament axis increases with contraction while the amount of stagger between adjacent sarcomeres decreases. To date, the discussion of this motion in the literature (1–2, 8, 11–12) has not considered the constraining role of the lateral attachment of the sarcomeres to the basal lamina and cuticle. This attachment maintains the staggered position and spacing of the columns of sarcomeres at rest. During

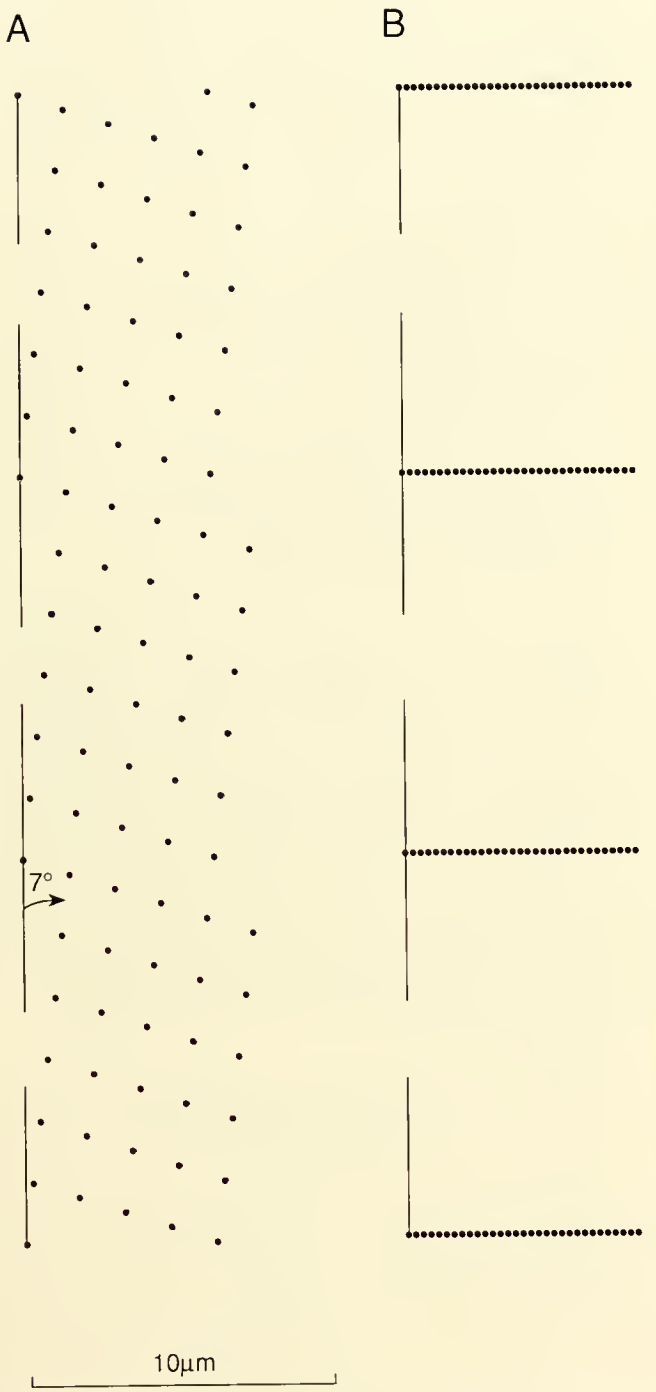

Figure 3. Surface view of nematode body wall muscle cell showing distribution of dense body attachment sites (dots) to the basal lamina: (A) with observed 7° oblique striation, and (B) rearranged as if it were cross-striated. The lines in the first column of sarcomeres indicate orientation of the thin filaments. Three complete sarcomeres are illustrated in each diagram. Longitudinal dimension is vertical. The pattern in (A) was drawn to scale from a surface view of *C. elegans* body-wall muscle photographed under phase optics published by Francis and Waterston (16). The same pattern is seen in radial sections of coelomyarian muscle.

the shortening of sarcomeres, the change in both stagger and striation angle must be governed by the elastic properties of the basal lamina and cuticle. Figure 3A illustrates the actual distribution of dense body attachment sites on the basal lamina. The system can be modeled by transferring the pattern of attachment sites onto a rubber membrane that has been previously stretched along the myofilament axis. The rubber membrane is thus equivalent to the basal lamina and cuticle under isometric tension. When the rubber membrane is allowed to shorten (equivalent to muscle contraction), the pattern width increases, the amount of stagger decreases, and the striation angle increases. Thus, the elastic properties of the membrane govern how the pattern is deformed during shortening. The previously hypothesized link between shearing and angle of striation in obliquely striated muscle (3) must, at least in nematodes, be the elastic basal lamina and cuticle to which the sarcomeres are attached.

What is the significance of the oblique arrangement of sarcomeres? We propose that it distributes the attachment sites more evenly over the basal lamina. Compare the distributions with sarcomere attachment sites displaced longitudinally (Fig. 3A) and arranged orthogonally as if cross-striated (Fig. 3B). With oblique striation, the sarcomere compression zones overlap, the attachment sites are spread apart, and a more even loading pattern is generated within the basal lamina. The lateral transmission of sarcomere contraction and the staggering of the sarcomere attachment sites should result in essentially continuous compressive loading of the adjacent cuticle and a smooth bend in the body tube.

Oblique striation has been described for muscles of platyhelminths, nematodes, gastrotrichs, nematomorphs, priapulids, pogonophora, chaetognathes, annelids, molluscs, brachiopods, and an echinoderm (3–7, 9), and dense bodies are an obvious feature in all taxa. A provocative question is to what extent the lateral transmission of contractile force and the even distribution of attachment sites on the basal lamina—discovered in nematode body wall muscles—are significant architectural features of other obliquely striated muscles.

Acknowledgments

We thank Scott Clark for providing the *lin-39* strains, Gwen Bollerup for collection of *Mermis*, Hella Prochaska for assistance with electron microscopy, and Bonny Milsome for the art work. Supported by grants from the Natural Sciences and Engineering Research Council of Canada to AHJB and from the Leo Leeser Foundation for Tropical Biology to CG.

Literature Cited

1. **Rosenbluth, J. 1972.** Obliquely striated muscle. Pp. 389–420 in *The Structure and Function of Muscle*, 2nd ed., vol. 1, part 1, G. H. Bourne, ed. Academic Press, New York.

2. **Knapp, M. F., and P. J. Mill. 1971.** The contractile mechanism in obliquely striated body wall muscle of the earthworm, *Lumbricus terrestris*. *J. Cell Sci.* **8:** 413–425.
3. **Lanzavecchia, G. 1977.** Morphological modulations in helical muscles (Aschelminthes and Annelida). *Int. Rev. Cytol.* **51:** 133–186.
4. **Toida, N., H. Kuriyama, N. Tashiro, and Y. Ito. 1975.** Obliquely striated muscle. *Physiol. Rev.* **55:** 700–756.
5. **Lanzavecchia, G., R. Valvassori, M. de Eguileor, and P. Lanzavecchia, Jr. 1979.** Three-dimensional reconstruction of the contractile system of the Nematomorpha muscle fiber. *J. Ultrastruct. Res.* **66:** 201–227.
6. **Candia Carnevali, M. D., A. Saita, and A. Fedrigo. 1986.** An unusual Z-system in the obliquely striated muscles of crinoids: three-dimensional structure and computer simulations. *J. Muscle Res. Cell Motil.* **7:** 568–578.
7. **Ward, S. M., J. M. Allen, and G. McKerr. 1992.** Physiology of obliquely striated muscle fibers within *Grillotia erinaceus* metacestodes (Cestoda: Trypanorhyncha). *Parasitology* **104:** 337–346.
8. **De Eguileor, M., R. Cotelli, R. Valvassori, M. Brivio, and L. Di Lernia. 1988.** Functional significance of intermediate filament meshwork in annelid helical muscles. *J. Ultrastruct. Mol. Struct. Res.* **100:** 183–193.
9. **Paniagua, R., M. Royuela, R. M. Garcia-Anchuello, and B. Fraile. 1996.** Ultrastructure of invertebrate muscle cell types. *Histol. Histopathol.* **11:** 181–201.
10. **Vigoreau, J. O. 1994.** The muscle Z band: lessons in stress management. *J. Muscle Res. Cell Motil.* **15:** 237–255.
11. **Prosser, C. L. 1991.** Animal Movement. Pp. 83–84 in *Neural and Integrative Animal Physiology*, C. L. Prosser, ed. Wiley-Liss, New York.
12. **Rosenbluth, J. 1967.** Obliquely striated muscle. III. Contraction mechanism of *Ascaris* body muscle. *J. Cell Biol.* **34:** 15–33.
13. **Rosenbluth, J. 1965.** Ultrastructural organization of obliquely striated muscle fibers in *Ascaris lumbricoides*. *J. Cell. Biol.* **25:** 495–515.
14. **Hope, W. D. 1969.** Fine structure of the somatic muscles of the free-living marine nematode *Deontostoma californicum* Steiner and Albin, 1933 (Leptosomatidae). *Proc. Helminthol. Soc.* **36:** 10–29.
15. **Hirumi, H., D. J. Raski, and N. O. Jones. 1971.** Primitive muscle cells of nematodes: morphological aspects of platymyarian and shallow coelomyarian muscles in two plant parasitic nematodes, *Trichodorus christiei* and *Longidorus elongatus*. *J. Ultrastruct. Res.* **34:** 517–543.
16. **Francis, G. R., and R. H. Waterston. 1985.** Muscle organization in *Caenorhabditis elegans:* localization of proteins implicated in thin filament attachment and I-band organization. *J. Cell Biol.* **101:** 1532–1549.
17. **Barstead, R. J., and R. H. Waterston. 1989.** The basal component of the nematode dense body is vinculin. *J. Biol. Chem.* **264:** 10177–10185.
18. **Francis, R., and R. H. Waterston. 1991.** Muscle cell attachment in *Caenorhabditis elegans*. *J. Cell Biol.* **114:** 465–479.
19. **Valvassori, R., M. de Eguileor, and G. Lanzavecchia. 1981.** Studies on the helical and paramyosinic muscle. VIII. Ultrastructural analysis of body wall muscles from *Mermis* sp. *J. Ultrastruct. Res.* **76:** 82–88.
20. **Harris, J. E., and H. D. Crofton. 1957.** Structure and function in nematodes: internal pressure and cuticular structure in *Ascaris*. *J. Exp. Biol.* **34:** 116–130.
21. **Niebur, E., and P. Erdös. 1991.** Theory of the locomotion of nematodes. Dynamics of undulatory progression on a surface. *Biophys. J.* **60:** 1132–1146.
22. **Gray, J., and H. W. Lissmann. 1964.** The locomotion of nematodes. *J. Exp. Biol.* **41:** 135–154.
23. **Price, M. G. 1991.** Striated muscle endosarcomeric and exosarcomeric lattices. *Adv. Struct. Biol.* **1:** 175–207.
24. **Wang, K., R. McCarter, J. Wright, J. Beverly, and R. Ramirez-Mitchell. 1993.** Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin-myosin composite filament is a dual-stage molecular spring. *Biophys. J.* **64:** 1161–1177.
25. **Small, J. V. 1995.** Structure-function relationships in smooth muscle: the missing links. *Bioessays* **17:** 785–792.
26. **Stromer, M. H. 1995.** Immunocytochemistry of the muscle cell cytoskeleton. *Microsc. Res. Techn.* **31:** 95–105.
27. **Moerman, D. G., and A. Fire. 1997.** Muscle: structure, function, and development. Pp. 417–470 in *C. elegans II*, D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
28. **Moulder, G. L., M. M. Huang, R. H. Waterston, and R. J. Barstead. 1996.** Talin requires β-integrin, but not vinculin, for its assembly into focal adhesion-like structures in the nematode *Caenorhabditis elegans*. *Mol. Biol. Cell* **7:** 1181–1193.
29. **Gans, C., and A. H. J. Burr. 1994.** Unique locomotory mechanism of *Mermis nigrescens*, a large nematode that crawls over soil and climbs through vegetation. *J. Morphol.* **222:** 133–148.
30. **Bartnik, E., M. Osborn, and K. Weber. 1986.** Intermediate filaments in muscle and epithelial cells of nematodes. *J. Cell Biol.* **102:** 2033–2041.
31. **Timoshenko, S., and G. H. MacCullough. 1940.** *Elements of Strength of Materials*, VanNostrand, New York.
32. **Clark, S. G., A. D. Chisholm, and H. R. Horvitz. 1993.** Control of cell fates in the central body region of *C. elegans* by the homeobox gene *lin-39*. *Cell* **74:** 43–55.
33. **Mackenzie, J. M., and H. F. Epstein. 1980.** Paramyosin is necessary for determination of nematode thick filament length *in vivo*. *Cell* **22:** 747–755.
34. **Brenner, S. 1974.** The genetics of *Caenorhabditis elegans*. *Genetics* **77:** 71–94.